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ABSTRACT

Photochemical degradation is an important process involved in the decay of macrophytes, but little is known on the response of heterotrophic bacteria to the chromophoric dissolved organic matter (CDOM) derived from different plant species. Here, we assessed the effect of photoaltered CDOM derived from Schoenoplectus californicus *and* Ruppia maritima *on the composition and production of a bacterial community from a productive subtropical coastal lagoon. Chromophoric dissolved organic matter from plants extracts was characterised by optical methods before and after exposure to natural full solar radiation. Afterwards, bacteria from the lagoon were added and incubated in these extracts for 5 h. We found that CDOM from the plant extracts underwent photoalteration after exposure to natural solar radiation, inducing shifts in the original bacterial community composition. In both macrophyte extracts, the bacterial community significantly changed and became dominated by two populations with distinctive morphology. The main enriched bacteria in both plant extracts were large filaments that made up to 99% of the community biovolume. In the* R. maritima *extract, another type of enriched bacteria was detected, consisting of large rods. 16S rDNA sequencing showed that the enriched bacterial populations belonged to* Exiguobacterium *sp. (filaments) and* Acinetobacter *sp. (rods). Morphotype identities were confirmed by fluorescence* in situ *hybridisation using specific probes targeting those taxa. Our results suggest that solar-induced photoalteration of plant material in this coastal lagoon results in the growth of opportunistic bacterial taxa.*

Key words: chromophoric dissolved organic matter, macrophytes, Exiguobacterium, Acinetobacter*, coastal lagoon.*

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INTRODUCTION

Dissolved organic matter (DOM) has different sources in aquatic ecosystems, *e.g.* allochthonous, from soil runoff, or autochthonous, from exudation and decomposition of primary producers. The relative contribution of DOM derived from each source will depend on the biological and physicochemical characteristics of the water body and its catchment. In particular, the hydrology of the systems is an important factor conditioning the source and availability of DOM. Allochthonous DOM differs from autochthonous in several optical (McKnight *et al*., 1994) and chemical characteristics (Benner, 2002). Allochthonous organic matter is usually more refractory to bacterial degradation (del Giorgio and Davis, 2003), probably due to the high proportion of chromophoric DOM (CDOM) consisting of humic substances. Differences in chemical composition of CDOM are thought to influence the structure of natural bacterial assemblages (Crump *et al*., 2003). From several studies assessing changes in the structure and activity of the bacterial community in relation to DOM (Cottrell and Kirchman, 2000; Elifantz *et al*., 2005; Alonso *et al*., 2009), it seems that even considering large taxonomic groups, bacteria differ in their capacity to use specific dissolved organic compounds. Therefore, qualitative changes in the DOM source might trigger a shift in bacterial community structure and activity.

The CDOM concentration in freshwater is usually higher than in marine water, owing to the high primary productivity of macrophytes and larger influence of the terrestrial catchment (Wetzel, 1992). Plant-derived DOM can enter ecosystems via photosynthates excretion or by plant lixiviation (Mann and Wetzel, 1996), which is considered a main source of DOM in many aquatic systems (Wetzel, 1992). In coastal lagoons, macrophytes can cover large littoral extensions; as a consequence, CDOM from plant-decaying material is one of the main carbon and nutrient source to these ecosystems (Conde *et al.*, 2000). During the decay process, photochemical degradation is important because small molecular weight substrates are made available for microbial growth (Wetzel, 2003). However, little is known on how photoaltered CDOM from different macrophyte species affects the bacterial community.

Alonso *et al*. (2013) recently showed that the addition of allochthonous carbon sources induces pronounced

shifts in the bacterial assemblage of a shallow coastal lagoon, while the amendment of nutrients such as nitrogen (N) or phosphorus (P) does not have a significant effect on it. These results suggest that bacterioplankton growth in this type of aquatic ecosystem would be predominantly limited by carbon and that this limitation would be more or less intense depending on the hydrology of the system (Alonso *et al*., 2013). The reason for this limitation is probably that during some phases of the lagoon hydrology, the main source of organic carbon for bacteria is macrophytes-derived DOM, which due to their chemical composition cannot be readily incorporated by bacteria for growth. In fact, we have previously shown that photoalteration of CDOM stimulates bacterioplankton growth and induces changes in community composition (Piccini *et al*., 2009). One of the current hypotheses about the possible carbon sources for bacterioplankton in this system is that UV-induced photoalteration of macrophytes-derived DOM increases carbon availability to sustain bacterial growth. Further, we expect that the photoproducts derived from different decaying macrophytes species will differ in their overall effect on the bacterial assemblage. Here, we determined whether photoaltered CDOM derived from two macrophytes species of a coastal lagoon, namely the California bulrush *Schoenoplectus californicus* (C.A. Mey.) Palla and the widgeongrass *Ruppia maritima* L. (Rodríguez-Gallego *et al*., 2010), is a relevant process shaping the heterotrophic bacterial community.

METHODS

Preparation of dissolved organic matter sources and experimental setup

To assess the effect of solar radiation on the different DOM sources, an *in situ* experiment was done in January during the austral summer in the freshwater zone of a brackish coastal lagoon (Laguna de Rocha, Uruguay, 34°837'S, 54°817'W) (Conde *et al*., 2000). Two different classes of DOM consisting of extracts from *S. californicus* (submerged stems) (SCD) and *R. maritima* (whole plants) (RMD) were selected. Samples were processed in sterile Milli-Q water (Millipore, Billerica, MA, USA) by homogenising the material for 10 min at room temperature using a Stomacher lab blender (Seward, Worthing, UK). Homogenates were centrifuged at 2000 x g and the supernatants were filtered through 0.2 µm polycarbonate filters (Millipore), previously soaked overnight in 10% HCl and rinsed with 20 mL of Milli-Q water. Absence of bacterial contamination in the resulting filtrate was checked by epifluorescence microscopy and 4',6-diamino-2-phenylindole (DAPI) counts on 0.2 µm filters (Porter and Feig, 1980).

The DOM extracts were filled into 100 mL spherical quartz bottles in triplicate (Piccini *et al*., 2009), and exposed to natural solar radiation for two days (8 h of effective solar exposure each day) in a circulating water bath at *in situ* temperature (24.5ºC), where they were completely submerged and manually rotated each hour. Optical properties of the filtrates were determined before (initial) and after exposure to solar radiation (16 h). In addition, dissolved organic carbon (DOC) concentration was measured before exposure of the DOM extracts.

Solar UV irradiance during exposure was measured *in situ* with an International Light radiometer (IL-1400A; International Light Technologies Inc., Peabody, MA, USA) consisting of the broadband SUL033/W (UV-A) and SUL240/W (UV-B) sensors. Incident radiation for UV-B and UV-A was integrated for the total exposure and corresponded to 5.2 J and 237.4 J cm^{-2}, respectively.

To assess the response of the *in situ* bacterial assemblage, each quartz bottle was inoculated with 10 mL of water collected from the freshwater zone of the lagoon to obtain a 1:10 dilution of the original lagoon community (T0) and thus to minimise the effect of predators. All quartz bottles were then incubated for 5 h under natural solar radiation. A set of 3 quartz bottles was used as control, which were filled with the natural microbial community, but without plant extracts. Incident radiation for UV-B and UV-A during this incubation corresponded to 2.1 J and 25 J cm^{-2}, respectively. Measurements of CDOM optical characteristics were repeated after the 5 h incubation.

Optical characterisation of chromophoric dissolved organic matter

For the analysis of DOC concentration and optical properties of CDOM, samples were filtered on the same day (within 6 h) through 0.2 µm polycarbonate filters (Millipore), previously soaked overnight in 10% HCl and rinsed with 20 mL of Milli-Q water (Millipore). Filtered water was stored in precombusted (500°C, 1 h) glass bottles (Teflon-capped) and kept in the dark at -20°C for DOC analysis or at 4°C for the optical characterisation of CDOM (absorption and fluorescence). The concentration of DOC (as non purgeable organic carbon) was measured using a high temperature catalytic oxidation method (Shimadzu TOC analyser model 5000; Shimadzu, Kyoto, Japan). Calibration of the instrument was done with potassium hydrogen phthalate (four points calibration curve). A consensus reference material (CRM) for DOC [batch 5 FS-2005:0.57 mg C L^{-1} provided by the Rosenstiel School of Marine and Atmospheric Science/Division of Marine and Atmospheric Chemistry (RSMAS/MAC), University of Miami] was run in parallel. Results differed from the CRM given value by 5% and the coefficient of variation was better than 2%.

Chromophoric dissolved organic matter absorbance was measured at room temperature in a double-beam spectrophotometer (Shimadzu UV1603; Shimadzu) between 280 and 750 nm using acid-cleaned 5 cm fused silica cuvettes (Suprasil® I) previously rinsed with Milli-Q

water (Millipore) and twice with the filtered sample. Milli-Q water (Millipore) of low DOC concentration (0.05 mg L^{-1}) was used as a reference. Absorption coefficients (a) were calculated as: a=2.303D/L, where D is absorbance and L is the pathlength of the cuvette in expressed in m. Correction for the scattering caused by particles was done by subtracting the absorbance value at 690 nm. For comparative purposes, we used the absorption value at 320 nm (a_{320}), which is sensitive to changes caused by both UV-B and UV-A radiation. The CDOM absorption at 320 nm was also normalised to the DOC concentration (a^*_{320}). Spectral slope (S) was calculated for each treatment before and after exposure for the ranges 275-295 nm (UV-B) and 350-400 nm (UV-A) (Helms *et al*., 2008). In addition, the ratio of both calculated slopes (S_R) was calculated to trace changes in CDOM related to photobleaching (Helms *et al*., 2008).

To infer changes in the dominant molecular size of CDOM, the ratio between the absorption coefficients at 250 and 365 nm (a_{250}:a_{365}) was used, where high ratios indicate a high proportion of small molecules and low ratios a high proportion of large molecules (De Haan, 1993). Samples for fluorescence measurements were filtered as for DOC and the filtrates were collected in 20 mL vials (acid washed, rinsed with Milli-Q water, and pre-combusted as above). Fluorescence (Fl) was measured at room temperature in an AMINCO Bowman Serie 2 spectrofluorometer (excitation at 355 nm, emission at 450, slit width of 5 nm) using a 1 cm quartz cuvette, prerinsed with Milli-Q water (Millipore) and with the filtered sample. Fluorescence standardisation was done by normalising the signal of the sample to the fluorescence of a solution of quinine sulfate 1 mg L^{-1} in 1 N H_2SO_4 defined as 1 quinine sulfate unit (QSU). Fluorescence was linear between 0.05 and 10 mg L^{-1} of quinine sulfate. To account for potential instrumental drift, separate quinine sulfate spectra were acquired on each day of measurement.

Bacterial abundance and biovolume

Samples (3 mL) were taken from each treatment bottle and fixed with 1% paraformaldehyde-PBS pH 7.2. On the same day, samples were filtered through polycarbonate 0.2 µm pore diameter filters (Millipore). Bacterial abundance (excluding filaments and large rods) was determined by epifluorescence microscopy and DAPI staining (final concentration 1 µg mL^{-1}) as previously described (Porter and Feig, 1980). The biovolume of filaments and large rods was determined at the beginning (T0) and the end of bacterial incubation (5 h) using the image analysis process and algorithms described by Massana *et al*. (1997). The cumulative length of filaments (µm mL^{-1}) in each sample and the corresponding biovolume were calculated according to Nedoma *et al*. (2001). Images were taken at 8 bit, resolution 1040×1388 pixels with a ×40 magnification resulting in a pixel length of ~0.466 µm. Image analyses and measurements of cells dimensions were done using J software (NIH, Bethesda, MD, USA).

Bacterial carbon production

Bulk bacterial activity was estimated from the incorporation of [^{3}H]-L-leucine (Amersham, Little Chalfont, UK) into bacterial biomass (Simon and Azam, 1989). Samples were taken from each of the triplicates at the initial time (T0) and at final time (5 h). Radiolabelled leucine (specific activity 63 Ci $mmol^{-1}$) was added at saturating concentrations (20 nM) (Piccini *et al*., 2009) to three subsamples and to one formaldehyde-killed control (3% final concentration) from each water treatment. These sub-samples were incubated in a water bath in the dark at *in situ* temperature (25ºC) for 1 h and then fixed with formaldehyde (3% final concentration). Macromolecule extraction was done with ice-cold trichloroacetic acid (TCA) (5% w/v) and ice-cold ethanol (80% v/v) according to Simon and Azam (1989). Measurements of [^{3}H]-L-leucine incorporation were done in a Beckman LS5000TD liquid scintillation counter (Beckman, Fullerton, CA, USA). Values were corrected for quenching (external standard method) and by subtraction of counts from the controls. Conversion of leucine incorporation to bacterial protein and carbon production (BCP) was done using the conversion factors described by Simon and Azam (1989).

Polymerase chain reaction amplification, cloning, and sequencing of 16S rRNA genes

16S rRNA gene clone libraries were constructed from fixed and filtered samples obtained from SCD and RMD incubations as described for bacterial abundance. Pieces of filter of approximately 6 mm^2 were cut out and used directly as a template for amplification by polymerase chain reaction (PCR) without previous DNA extraction (Kirchman *et al*., 2001). Polymerase chain reaction amplification with the general bacterial primers GM3F and GM4R (Muyzer *et al*., 1995) was done with a Mastercycler (Eppendorf, Hamburg, Germany) using the following conditions: an initial denaturation step of 5 min at 94°C, 1 min of denaturation at 94°C, 1.5 min of annealing at 48°C, and 2 min of primer extension at 72°C. This cycle was repeated 30 times, followed by a final step of 2 min at 72°C. The PCR products were checked by agarose (1%, wt/vol) gel electrophoresis and the amplified 16S rRNA gene fragments were purified with a QIAquick PCR purification kit (QIAGEN, Hilden, Germany). The purified amplicons were inserted into the pGEM vector (PGEM-T cloning kit; Promega, Fitchburg, WI, USA) and cloned into competent *Escherichia coli* cells delivered with the kit as described by the manufacturer. White clones were picked and plasmids were extracted with a QIAprep spin miniprep kit (QIAGEN)

according to the manufacturer's specifications. Ninety-six clones from each library were screened for correctly sized inserts by PCR using the vector primers (M13F and M13R) and further agarose gel electrophoresis of the amplicons (1%), and proved to be positive. Plasmids partial sequences were obtained from these positive clones using the primer M13F on an ABI PRISM 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA). After analysing the partial sequences, 48 clones from each library (SCD and RMD) were selected for complete sequencing of the 16S rRNA gene using the primers M13R and GM1. Sequences were assembled from three partial sequences using the software Sequencher (Gene Codes, Ann Arbor, MI, USA). All sequences were subsequently analysed by BLAST (Altschul *et al*., 1990) to identify their closest relatives and to establish tentative phylogenetic affiliations. Phylogenetic analyses were performed using ARB software (Ludwig *et al*., 2004). For the reconstruction of a phylogenetic tree depicting the affiliations of newly obtained sequences, only nearly complete 16S rRNA sequences were considered. Maximum-parsimony, neighbour-joining, and maximum-likelihood analyses were performed. The branching patterns of the resulting trees were compared and a consensus tree was constructed that showed bifurcations only if branchings were stable in the majority of analyses. Multifurcations were introduced if tree topology could not be unambiguously resolved.

Double fluorescence *in situ* hybridisation

After sequencing of clones from SCD and RMD treatments, a search for probes targeting the identified bacterial groups was conducted in the probeBase website. Selected probes were used in a double FISH, combining CARD and monolabeled probes according to the protocol described by Pernthaler *et al*. (2004). Filters used for double FISH were the same as for bacterial abundance determination and clone libraries construction. Briefly, a first hybridisation using the peroxidase-conjugated probe LGC354A (Meier *et al*., 1999) for low guanine-cytosine (GC) content Gram-positive bacteria was done including the catalysed reporter deposition step, but the hybridised filter pieces were not counterstained with DAPI. Instead, they were incubated for 20 min at room temperature in 0.01M HCl to inactivate the horseradish peroxidase and then a second hybridisation was run using the monolabeled S-G-Acin-0652-a-A-18 probe (Oerther *et al*., 1998) for *Acinetobacter* spp. conjugated to Cy3. Formamide concentrations in the hybridisation buffers were 55% for LGC354A and 40% in the case of S-G-Acin-0652-a-A-18 probe.

Statistical analyses

Unpaired t-tests were used to test for significant differences in CDOM optical parameters, bacterial abundance, and bacterial biomass at the beginning and the end of the experiments. The comparison of the effect of treatments and time on BCP was done with a two-way ANOVA and Bonferroni *post-hoc* test. For all parameters, the average values were calculated from the three replicates in each treatment. Differences were considered significant when $P<0.05$. All statistical analyses were run using GraphPad Prism version 5.0 for Mac OS X, GraphPad Software (GraphPad, San Diego, CA, USA).

RESULTS

Photoalteration of chromophoric dissolved organic matter

The absorption coefficient at 320 nm (a_{320}) of the macrophyte extracts decreased after 16 h exposure (unpaired t-test, $P<0.01$) (Tab. 1). Similarly, the spectral slopes and the S_R for each treatment increased in the UV-B range after irradiation of both plant extracts (unpaired t-test, $P<0.01$). The a_{250}:a_{365} ratios from SCD before exposure to solar radiation were higher than the observed for RMD (Tab. 1). After exposure to solar radiation, the a_{250}:a_{365} ratio increased in both extracts. Thus, the relative molecular size of CDOM decreased by ca. 10% in SCD (unpaired t-test, $P<0.05$) and by ca. 17% in RMD (unpaired t-test, $P<0.05$) extract (Tab. 1). No further significant change in CDOM optical characteristics was observed after the 5 h incubation (data not shown).

Bacterial community response

When the bacterial community was analysed in both plant extracts after 5 h incubation, changes in cell size distribution were detected, namely, the mean cell volume was significantly larger (unpaired t-test, $P<0.05$) than that of the original natural bacterial community (Figs. 1 and 2). In the bacterial community incubated without plant extracts, the mean cell volume was the same as in T0 (data not shown). In SCD, the bacterial community was dominated by large filaments of ca. 100 μm length and ca. 5 μm wide, with a mean volume of $6.19\pm2.72 \times 10^6$ μm^3 mL^{-1}, corresponding to 97.7% of the whole community biovolume (Fig. 2C). Filaments were also present in RMD, where they comprised 90.6% of the whole community biovolume. In addition, in the RMD treatment large rods with an average cellular volume of 6.5 ± 2.37 μm^3 and representing 8.7% of the bacterial biovolume were found (Fig. 2D). These rods were also found in the SCD treatment, but at lower abundance. Both enriched bacterial populations (large filaments and rods) were undetectable at the initial time (Fig. 2A and 2B). Furthermore, the abundance of bacteria that were not large rods nor filaments present in both treatments did not show significant changes during incubation (unpaired *t*-test, $P>0.05$) (Fig. 3), suggesting that the newly developed bacterial populations responded to the photoaltered plant-derived DOM,

whereas the rest of the community did not. When the bulk BCP was assessed, an increase was observed after 5 h in both treatments, but it was significant only in the case of the RMD extract (unpaired t-test, P<0.05) (Fig. 4).

To identify the dominant bacterial populations, clone libraries of the 16S rRNA gene were constructed from SCD and RMD samples and almost complete (ca. 1500 bp) 16S rDNA sequences were obtained from several clones. Pairwise comparison against Genebank database and phylogenetic analyses using ARB (Ludwig *et al*., 2004) showed that 20.8% of the clones proved to be positive from SCD treatment and harboured sequences ≥99% identical to several strains of *Exiguobacterium acetylicum* and 35.4% to *Acinetobacter* spp. (Fig. 5A). In contrast, 18.8% of sequences obtained from positive clones belonging to RMD clone library showed 96 to 99% identity to *Exiguobacterium* spp. and 21% showed 100% similarity with *Acinetobacter* sp., being *A. bayly* the closest described phylotype (Fig. 5B). To confirm the identity of the enriched bacteria, oligonucleotide probes targeting the obtained ribosomal sequences (Acin0659A for *Acinetobacter* and LGC354A for *Exiguobacterium*) were applied in FISH assays. Both probes hybridised to the expected bacterial morphotypes (*i.e.* rods and filaments, respectively). In the case of Acin0659A-hybridised cells, abundance in SCD and RMD treatments were 1.48±0.34 and 2.52±3.03 x10^5 cells mL^{-1}, respectively. In RMD, where they were more abundant, Acin0659A-hybridised cells increased from 0.11% of the total bacterial community at the beginning of the experiment (or in the control) to >11% at the end of the incubation (Fig. 5C).

DISCUSSION

In this work, we found that solar radiation caused photobleaching of the macrophyte-derived CDOM as indicated by changes in its optical properties (a_{320}, Fl, and spectral slopes) (Kirk, 1994; McKnight *et al*., 2001; Helms *et al*., 2008). Considering that CDOM is heterogeneous in nature, only a small portion may be chromophoric and thus responsible for photochemical reaction (Osburn *et al*., 2001). The changes in optical parameters observed for the macrophyte extracts after incubation (a_{320}, spectral slopes and S_R) confirmed the photobleaching of CDOM. According to the a_{250}:a_{365} ratios measured before incubation, CDOM from SCD extract showed to be more labile than RMD, however, after exposure to solar radiation, these

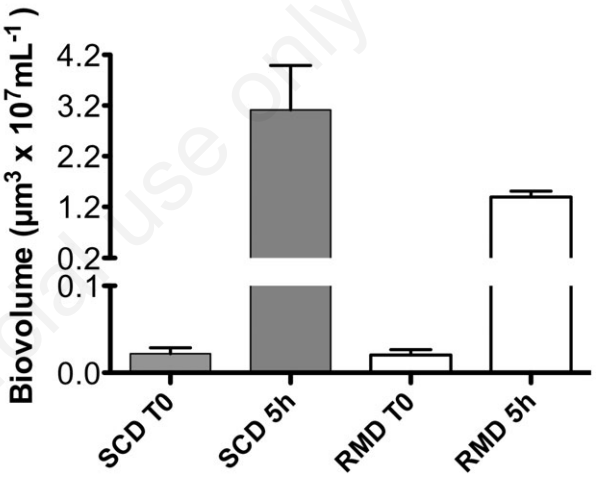

Fig. 1. Average bacterial volumes and standard deviations (in μm^3 per mL x10^7) calculated at the beginning (T0) and at the end (5 h) of the incubation in both plant treatments. SCD=*S. californicus* dissolved organic matter; RMD=*R. maritima* dissolved organic matter.

Tab. 1. Water optical characteristics for both plant extracts before and after exposure to solar radiation and initial dissolved organic carbon concentration.

	SCD		RMD	
	Initial (SD)	16 h (SD)	Initial (SD)	16 h (SD)
DOC (mg L^{-1})	99.80	ND	67.90	ND
a_{320} (m^{-1})	80.73 (0.20)*	51.90 (8.98)*	54.88 (0.49)*	38.53 (4.99)*
Fl (mg L^{-1})°	4.66 (0.02)	2.38 (0.59)	2.49 (0.03)	2.38 (0.85)
S (275-295 nm)	0.009 (0.001)*	0.015 (0.0001)*	0.003 (0.00)*	0.010 (0.001)*
S (350-400 nm)	0.024 (0.00)	0.020 (0.001)	0.018 (0.001)	0.016 (0.002)
S_R	0.36 (0.00)	0.77 (0.002)	0.17 (0.001)	0.60 (0.005)
a_{250}:a_{365}	4.87 (0.001)*	5.31 (0.24)*	2.95 (0.01)*	3.47 (0.13)*

SCD, dissolved organic matter from S. californicus*; RMD, dissolved organic matter from* R. maritima*; SD, standard deviation; DOC, dissolved organic carbon; ND, not determined;* a_{320}*, absorption coefficients at 320 nm; Fl, fluorescence;* S*, spectral slopes calculated from the absorption spectra before (initial) and after sunlight exposure (16 h);* S_R*, ratio of calculated slopes;* a_{250}:a_{365}*, ratio between the absorption coefficients at 250 and 365 nm. °Expressed as quinine sulphate units (QSU), where 1 QSU=1 µg L^{-1} of quinine sulphate; *significant differences between initial time and after 16 h incubation within each treatment at P< 0.05.*

ratios increased in both extracts, indicating that the bioavailability of DOM from the two plant sources was enhanced by exposure to solar radiation and photoalteration. The differences in optical characteristics found between both plants may reflect differences in their chemical composition for these species (Farjalla *et al*., 2001).

Previous studies have shown that sedimentary organic matter in Laguna de Rocha is mainly derived from the macrophyte community (Conde and Sommaruga, 1999) and two of the most abundant plant species in this lagoon are *S. californicus* and *R. maritima* (Rodríguez-Gallego *et al*., 2010). Furthermore, Piccini *et al*. (2009) showed that CDOM photoalteration in Laguna de Rocha stimulates bacterioplankton activity and induces changes in bacterioplankton community composition in few hours, suggesting that photochemical processes are important for the availability of the organic matter derived from macrophytes. In the present study, we found an increase in bacterial biovolume and BCP after 5 h incubation in both treatments, implying that the measured bacterial production could be mostly due to the enriched bacterial populations. The development of bacterial morphotypes with cell sizes ca. 100 times larger than the rest of the community (for the case of SCD) suggests that these two populations of microorganisms exhibiting high growth rates rapidly incorporated the available organic carbon. We cannot rule out the possibility that the large filaments and rods not only developed because they reacted fast to the DOM amendment, but also because they are more resistant to photooxidative stress than the rest of the community. For example, photoproducts such as hydrogen peroxide are known to cause cell oxidative stress (Scully *et al*., 1996). It was outside the scope of this study, however, to measure the production of reactive oxygen species (ROS) during the experiment.

According to the similarity search, the big rod morphotypes were *Acinetobacter*-related bacteria closely related to the strain ADP1 (Vaneechoutte *et al*., 2006). It has been described that this strain has the capability to accumulate polyphosphate (PolyP) grains in their cytoplasm

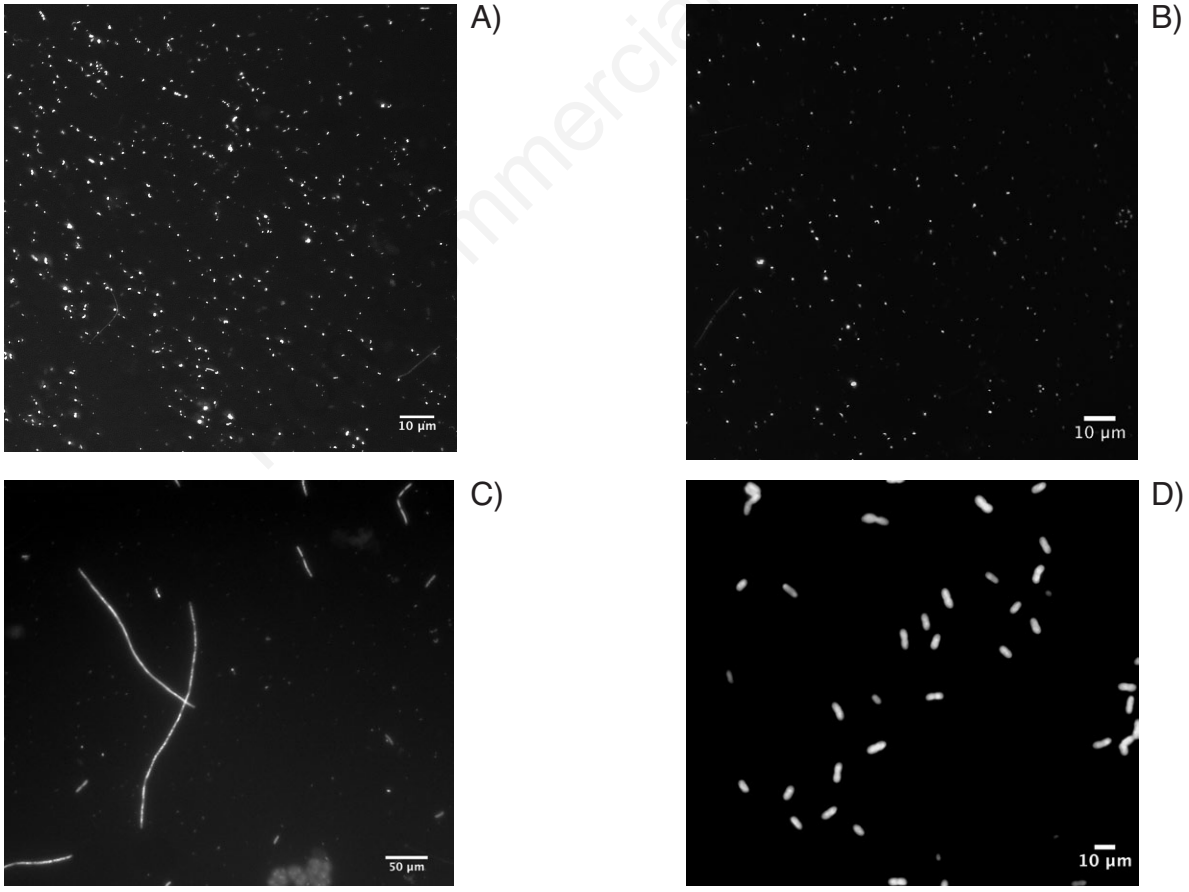

Fig. 2. Bacterial morphotypes found in A) *S. californicus* dissolved organic matter (SCD) treatment at T0 (DAPI stain) and B) *R. maritima* dissolved organic matter (RMD) treatment at T0 (DAPI stain). C) Filaments detected in SCD (DAPI stain) at 5 h; D) RMD hybridised with Acin0659A probe at 5 h.

under aerobic conditions and to use them later as a P source (Trelstad *et al*., 1999). Interestingly, microscopic examination of Acin0659A-hybridised cells showed the presence of yellow intracellular grains under UV excitation resembling PolyP (data not shown). The staining of bacterial PolyP granules by DAPI has been previously reported (Tijssen *et al*., 1982). In a study using Mediterranean seawater, Sebastián *et al*. (2012) found that some heterotrophic bacterial cells accumulate PolyP when incubated in phosphate-amended mesocosms. These authors proposed the existence of opportunistic bacteria (opportunitrophs) such as *Gammaproteobacteria* having high P requirements and able to accumulate phosphate inside the cell when this nutrient is available. The finding of fast-growing, PolyP-accumulating cells of the *Acinetobacter* genus in our experiments suggest that plant-derived DOM extracts were rich in phosphorus and allowed for the enrichment of opportunistic bacterial populations.

An interesting finding of the present study was the development of large bacterial filaments in the DOM extracts. The formation of bacterial filaments has been also reported in several studies, mainly associated to heavy predation by heterotrophic nanoflagellates (Sommaruga and Psenner, 1995; Corno and Jürgens, 2006). Since in our experiment the presence of predators was reduced by dilution of the inoculums and not by filtration, it is possible that their presence would induce changes in bacterial morphology, in spite of the short incubation times (5 h). The identification of such filaments by cloning and sequencing of the 16S rRNA genes revealed that the filaments belonged to *Exiguobacterium* genus. These bacteria have been found in diverse environments (Vishnivetskaya *et al*., 2000; Brambilla *et al*., 2001; Fruhling *et al*., 2002; Kim *et al*., 2005). This genus has been isolated from several habitats over a wide temperature range (-12 to 55°C) (Rodríguez and Tiedje, 2007) and it even survives to the exposure to UV-C radiation (Ponder *et al*., 2005). Some species of *Exiguobacterium* have also high levels of catalase and oxidase enzymes to minimise oxidative stress (Kim *et al*., 2005). This trait is probably important because ROS such as hydrogen peroxide are found among photoproducts from plant-derived DOM (Farjalla *et al*., 2001). Thus, the enrichment of an *Exiguobacterium* population with anti-ROS potential within a bacterial assemblage exposed to UVR would not be surprising. Further, it has been shown that natural UV radiation induces filamentation in lake bacterial assemblages (Corno *et al*., 2008; Modenutti *et al*. 2010). The exposure of bacteria to UV radiation could cause mutations in the *ftsZ* gene, which encodes for a protein involved in early stages of septum formation (Errington *et al*., 2003). For example, Feucht and Errington (2005) demonstrated that mutations of this gene induce filamentation in *Bacillus subtilis*. Interestingly, although the reported strains of *Exiguobacterium* are usually rods, the formation of filaments has been observed in response to temperatures as low as -6°C with an increase in both cell length and width (Vishnivetskaya *et al*., 2007). Overall, the facts discussed above indicate that *Exiguobacterium* filaments detected in our study might be a combined response to solar radiation, increased DOM availability, presence of predators, and phenotypic

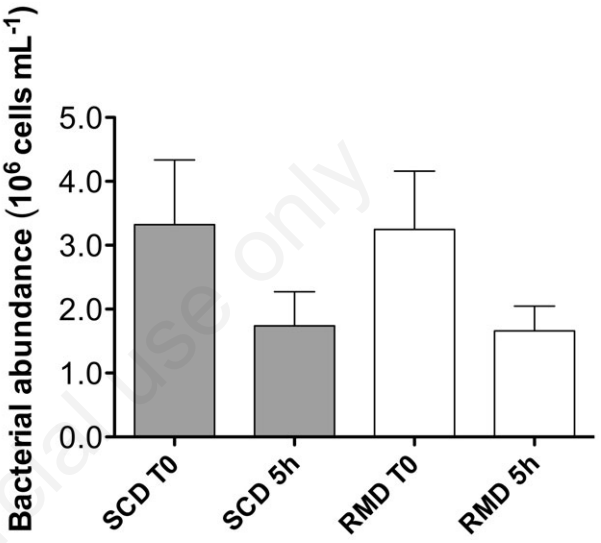

Fig. 3. Average bacterial abundance and standard deviations at the beginning (T0) and at after 5 h incubation in both plant treatments. Data do not include the abundance of large rods and filaments. SCD=dissolved organic matter from *S. californicus*; RMD=dissolved organic matter from *R. maritima*.

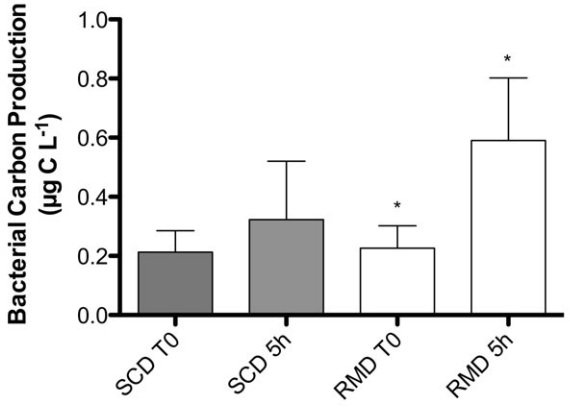

Fig. 4. Bacterial carbon production (BCP) measured in *S. californicus* dissolved organic matter (SCD) and *R. maritima* dissolved organic matter (RMD) treatments, at the beginning (T0) and after 5 h incubation. Asterisks indicate the treatments where significant differences were found between initial and final BCP.

plasticity of the genus. However, owing to our experimental design, we cannot establish whether the appearance of large filamentous bacterial populations is a response naturally occurring in the environment or an artifact.

CONCLUSIONS

Overall, the results of the present study suggest that loading of DOM from plant origin might be a relevant source of carbon for bacterial populations in the freshwater zone of coastal lagoons. Further, due to the opportunistic-like behaviour of *Acinetobacter* and *Exiguobacterium* genera, they would play major role in the mineralisation of plant material after photochemical breakdown.

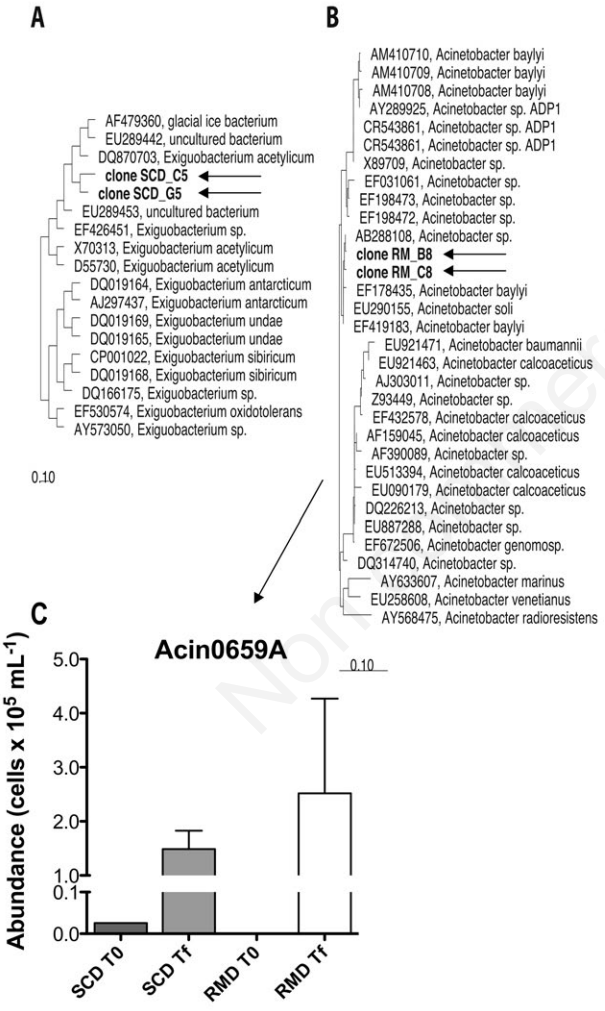

Fig. 5. Phylogenetic trees showing the affiliation of 16S rRNA gene sequences obtained from clones G5 and C5 of *S. californicus* extract (A) and B8 and C8 of *R. maritima* extract (B). Only nearly complete sequences are depicted. Arrows indicate the position of sequenced clones. C) Abundance of Acin0659A-hybridised cells (by fluorescence *in situ* hybridisation) in both plant treatments at the beginning (T0) and the end (Tf) of incubations. Bar, 10% estimated sequence divergence.

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REFERENCES

Alonso C, Zeder M, Piccini C, Conde D, Pernthaler J, 2009. Ecophysiological differences of betaproteobacterial populations in two hydrochemically distinct compartments of a subtropical lagoon. Environ. Microbiol. 11:867-876.

Alonso C, Piccini C, Unrein F, Bertoglio F, Conde D, Pernthaler J, 2013. Environmental dynamics as a structuring factor for microbial carbon utilization in a subtropical coastal lagoon. Front. Microbiol. 4:14.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Benner R, 2002. Chemical composition and reactivity, p. 59-90. In: D. Hansell and C. Carlson (eds.), Biogeochemistry of marine dissolved organic matter. Academic Press.

Brambilla E, Hippe H, Hagelstein A, Tindall BJ, Stackebrandt E, 2001. 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antartica. Extremophiles 5:23-33.

Conde D, Aubriot L, Sommaruga R, 2000. Changes in UV penetration associated with marine intrusions and freshwater discharge in a shallow coastal lagoon of the Southern Atlantic Ocean. Mar. Ecol.-Prog. Ser. 207:19-31.

Conde D, Sommaruga R, 1999. A review of the state of limnology in Uruguay, p. 1-31. In: J. Gopal and R. Wetzel (eds.), Limnology in developing countries. International Scientific Publ.

Corno G, Caravati E, Callieri C, Bertoni R, 2008. Effects of predation pressure on bacterial abundance, diversity, and size-structure distribution in an oligotrophic system. J. Limnol. 67:107-119.

Corno G, Jürgens K, 2006. Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. Appl. Environ. Microb. 72:78-86.

Cottrell MT, Kirchman DL, 2000. Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high- molecular weight dissolved organic matter. Appl. Environ. Microb. 66:1692-1697.

Crump BC, Kling GW, Bahr M, Hobbie JE, 2003. Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. Appl. Environ. Microb. 69:2253-2268.

De Haan H, 1993. Solar UV-light penetration and photodegradation of humic substances in peaty lake water. Limnol. Oceanogr. 38:1072-1076.

Del Giorgio PA, Davis J, 2003. Patterns in dissolved organic matter lability and comsumption across aquatic ecosystems, p. 399-424. In: S.E.G. Findlay and R.L. Sinsabaugh (eds.),

Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press.
Elifantz H, Malmstrom RR, Cottrell MT, Kirchman DL, 2005. Assimilation of polysaccharides and glucose by major bacterial groups in the Delaware Estuary. Appl. Environ. Microb. 71:7799-7805.
Errington J, Daniel RA, Scheffers DJ, 2003. Cytokinesis in bacteria. Microbiol. Mol. Biol. R. 67:52-65.
Farjalla VF, Anesio AM, Bertilsson S, Granéli W, 2001. Photochemical reactivity of aquatic macrophyte leachates: abiotic transformations and bacterial response. Aquat. Microb. Ecol. 24:187-195.
Feucht A, Errington J, 2005. ftsZ mutations affecting cell division frequency, placement and morphology in *Bacillus subtili*s. Microbiology 151:2053-2064.
Fruhling A, Schumann P, Hippe H, Straubler B, Stackebrandt H, 2002. *Exiguobacterium undae* sp. nov. and *Exiguobacterium antarcticum* sp. nov. Int. J. Syst. Evol. Micr. 52:1171-1176.
Helms JR, Stubbins A, Ritchie JD, Minor EC, Kieber DJ, Mopper K, 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol. Oceanogr. 53:955-969.
Kim IG, Lee MH, Jung SY, Song JJ, Oh TK, Yoon JH, 2005. *Exiguobacterium aestuarii* sp. nov. and *Exiguobacterium marinum* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. Int. J. Syst. Evol. Micr. 55:885-889.
Kirchman DL, Yu LY, Fuchs BM, Amann R, 2001. Structure of bacterial communities in aquatic systems as revealed by filter PCR. Aquat. Microb. Ecol. 26:13-22.
Kirk JTO, 1994. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, New York: 662 pp.
Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar, Buchner A, Lai T, Steppi S, Jobb G, Förster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, König A, Liss T, Lüssmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A, Schleifer KH, 2004. ARB: a software environment for sequence data. Nucleic Acids Res. 32:1363-1371.
Mann CJ, Wetzel RG. 1996. Loading and utilization of dissolved organic carbon from emergent macrophytes. Aquat. Bot. 53:61-72.
Massana R, Gasol JM, Bjørnsen PK, Black-Burn N, Hagstrom A, Hietanen S, Hygum BH, Kuparinen J, Pedros-Alio C, 1997. Measurement of bacterial size via image analysis of epifluorescence preparations: description of an inexpensive system and solutions to some of the most common problems. Sci. Mar. 61:397-407.
McKnight DM, Andrews ED, Spulding SA, Aiken GR, 1994. Aquatic fulvic acids in algal-rich antarctic ponds. Limnol. Oceanogr. 39:1972-1979.
McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Anderson DT, 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol. Oceanogr. 46:38-48.
Meier H, Amann R, Ludwig W, Schleifer K, 1999. Specific oligonucleotide probes for in situ detection of a major group of Gram-positive bacteria with low DNA G+C content. Syst. Appl. Microbiol. 22:186-196.
Modenutti B, Balseiro E, Corno G, Callieri C, Bertoni R, Caravati E, 2010. Ultraviolet radiation induces filamentation in bacterial assemblages from North Andean Patagonian lakes. Photochem. Photobiol. 86:871-881.
Muyzer G, Teske A, Wirsen CO, Jannasch HW, 1995. Phylogenetic relationship of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Arch. Microbiol. 164:165-172.
Nedoma J, Vrba J, Hanzl T, Nedbalova L, 2001. Quantification of pelagic filamentous microorganisms in aquatic environments using the line-intercept method. FEMS Microbiol. Ecol. 38:81-85.
Oerther DB, Danalewich J, Dulekurgen E, Leveque E, Freedman DL, Raskin L, 1998. Bioaugmentation of sequencing batch reactors for biological phosphorous removal: comparative rRNA sequence analysis and hybridization with oligonucleotide probes. Wa. Sci. Technol. 37:469-473.
Osburn CL, Zagarese H, Morris D, Hargreaves B, Cravero W, 2001. Calculation of spectral weighting functions for the solar photobleaching of chromophoric dissolved organic matter in temperate lakes. Limnol. Oceanogr. 46:1455-1467.
Pernthaler A, Pernthaler J, Amann R, 2004. Sensitive multicolour fluorescence in situ hybridization for the identification of environmental organisms, p. 711-726. In: G.A. Kowalchuk, F.J. De Bruijn, I.M. Head, A.D.L. Akkermans and J.D. van Elsas (eds.), Molecular microbial ecology manual. Kluwer Academic Publ.
Piccini C, Conde D, Pernthaler J, Sommaruga R, 2009. Alteration of chromophoric dissolved organic matter by solar UV radiation causes rapid changes in bacterial community composition. Photochem. Photobio. Sci. 8:1321-1328.
Ponder MA, Gilmour SJ, Bergholz PW, Mindock CA, Hollingsworth R, Thomashow MF, Tiedje JM, 2005. Characterization of potential stress responses in ancient Siberian permafrost psychroactive bacteria. FEMS Microbiol. Ecol. 53:103-115.
Porter KG, Feig YS, 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25:943-948.
Rodríguez DF, Tiedje JM, 2007. Multi-locus real-time PCR for quantitation of bacteria in the environment reveals *Exiguobacterium* to be prevalent in permafrost. FEMS Microbiol. Ecol. 59:489-499.
Rodríguez-Gallego L, Meerhoff E, Clemente JM, Conde D, 2010. Can ephemeral proliferations of submerged macrophytes influence zoobenthos and water quality in coastal lagoons? Hydrobiologia 646:253-269.
Scully NM, Lean DRS, McQueen DJ, Cooper WJ, 1996. Hydrogen peroxide formation: the interaction of ultraviolet radiation and dissolved organic carbon in lake waters along a 43-75ºN gradient. Limnol. Oceanogr. 41:540-548.
Sebastián M, Pitta P, Gonzalez JM, Thingstad TF, Gasol JM, 2012. Bacterioplankton groups involved in the uptake of phosphate and dissolved organic phosphorus in a mesocosm experiment with P-starved Mediterranean waters. Environ. Microbiol. 14:2334-2347.
Simon M, Azam F, 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol.-Prog. Ser. 51:201-213.
Sommaruga R, Psenner R, 1995. Permanent presence of gra-

zing-resistant bacteria in a hypertrophic lake. Appl. Environ. Microb. 61:3457-3459.

Tijssen JP, Beekes HW, Van SJ, 1982. Localization of polyphosphates in *Saccharomyces fragilis*, as revealed by 4', 6-diamidino-2-phenylindole fluorescence. Biochim. Biophys. Acta 721:394-398.

Trelstad PL, Purdhani P, Geidorfer W, Hillen W, Keasling JD, 1999. Polyphosphate kinase of *Acinetobacter* sp. strain ADP1: purification and characterization of the enzyme and its role during changes in extracellular phosphate levels. Appl. Environ. Microb. 65:3780-3786.

Vaneechoutte M, Young DM, Ornston LN, De Baere T, Nemec A, Van Der Reijden T, Carr E, Tjernberg I, Dijkshoorn L, 2006. Naturally transformable *Acinetobacter* sp. strain ADP1 belongs to the newly described species *Acinetobacter baylyi*. Appl. Environ. Microb. 72:932-936.

Vishnivetskaya TA, Kathariou S, McGrath J, Gilichinsky D, Tiedje JM, 2000. Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. Extremophiles 4:165-173.

Vishnivetskaya TA, Siletzky R, Jefferies N, Tiedje JM, Kathariou S, 2007. Effect of low temperature and culture media on the growth and freeze-thawing tolerance of *Exiguobacterium* strains. Cryobiology 54:234-240.

Wetzel RG, 1992. Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic matter in freshwater ecosystems. Hydrobiologia 229:181-198.

Wetzel RG, 2003. Solar radiation as an ecosystem modulator, p. 3-18. In: E.W. Helbling and H. Zagarese (eds.), UV Effects in aquatic organisms and ecosystems. RSC Publ.